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Evidence in Gabon for an Intrafamilial Clustering With Mother-to-Child and Sexual Transmission of a New Molecular Variant of Human T-Lymphotropic Virus Type-II Subtype B

Philippe Tuppin, Antoine Gessain, Mirdad Kazanji, Renaud Mahieux, Jean-Yves Cosnefroy, Fredj Tekaia, Marie-Claude Georges-Courbot, Alain Georges, and Guy de Thé

Unité d'Epidémiologie des Virus Oncogènes (P.T., A.G., M.K., R.M., G.T.) and Service d'Informatique Scientifique (F.T.), Institut Pasteur, Paris, France; Centre International de Recherches Médicales (CIRM), Franceville, Gabon (J.-Y.C., M.-C.G.-C., A.G.)

Following the observation of an HTLV-II seropositive 60-year-old woman living in Gabon (Central Africa), a serologic and molecular study of her family members was conducted in an attempt to determine the duration of the HTLV-II infection and the modes of transmission of the virus. Among 41 family members, five were HTLV-I seropositive and 7 exhibited specific HTLV-II antibodies in their sera as demonstrated by high immunofluorescence titers on C19 cells and/or specific Western-blot pattern. The second husband of the index case and two of his sisters were infected by the virus, suggesting the presence of HTLV-II in this family over two generations. Sequence analysis of an amplified fragment of 172 nucleotides within the gp21 of the env region (6469–6640) of four HTLV-II infected individuals revealed a new HTLV-II molecular variant of the subtype b diverging from the prototypes NRA and G12 by seven (4.1%) and five (2.9%) bases substitutions, respectively. Molecular analysis of the total *env* gene (1462 bp) and fragments of the *pol* and pX regions confirmed that this new African variant was the most divergent HTLV-II subtype b yet described, exhibiting 2.3% of nucleotide substitutions in the *env* gene (33 bases) as compared to the two HTLV-II b prototypes. These data demonstrate, for the first time in Africa, intrafamilial both mother-to-child transmission and sexual transmission between spouses of an HTLV-II b molecular variant, and also suggest that this virus has been present in Gabon for a long period of time.

KEY WORDS: HTLV-II, molecular variant, familial transmission, Africa

INTRODUCTION

The origin and routes of the worldwide dissemination of the human and simian T-cell lymphotropic retroviruses (HTLV-I [Poiesz et al., 1980], HTLV-II [Kalyanaraman et al., 1982], STLV-I [Miyoshi et al., 1982], STLV-II [Chen et al., 1994], and the recently isolated PTLV-L [Goubau et al., 1994] and STLV-PP1664 [Liu et al., 1994]) remain controversial. Human T-cell leukemia virus type II (HTLV-II) was isolated originally in 1982, and two molecular prototypes have now been described: subtype a (MO) [Shimotohno et al., 1985] and subtype b (NRA) [Lee et al., 1993], differing from each other by 3–6% at a nucleotide level, depending on the genes studied [Dube et al., 1993; Hall et al., 1992; Switzer et al., 1995].

HTLV-II is known to be highly endemic among disparate native New World Amerindian tribes, including, in the United States, the Navajo and Pueblo in New Mexico and the Seminole in Florida [Hjelle et al., 1993; Levine et al., 1993], as well as the Guayami in Panama [Lairmore et al., 1990; Pardi et al., 1993], the Cayapo and Kraho in Brazil [Maloney et al., 1992], the Wayu in Colombia [Ijichi et al., 1993], and the Tobas and Mataccos in Argentina [Biglione et al., 1993; Ferrer et al., 1993]. In the Western world, HTLV-II infection takes an epidemic course in intravenous drug users (IVDU) in the United States [Lee et al., 1989] and to a lesser extent in Europe [Soriano et al., 1993; Zella et al., 1990]. Both molecular subtypes have been found in Amerindians and in IVDU populations, but the subtype b, mostly prevalent in the Amerindian groups, is often referred to as the Paleo-Indian strain [Dube et al., 1993; Hjelle et al., 1993]. These data led to the view that HTLV-II was a "new world virus" brought from Asia to

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Address reprint requests to Pr. Guy de Thé, Unité d'Epidémiologie des Virus Oncogènes, Institut Pasteur, 28 rue du Dr Roux, 75724 Paris cedex 15.

the Americas by migration of the original mongoloid settlers through the Behring Strait some 10,000–40,000 years ago [Dube et al., 1993; Maloney et al., 1992]. Furthermore, consistent with this hypothesis, HTLV-II infections were reported recently in Mongolia [Hall et al., 1993a].

However, sporadic serological infection with HTLV-II has been described recently in West Africa [Bonis et al., 1994; Gessain et al., 1993] and in Central Africa [Delaporte et al., 1992; Dube et al., 1994; Goubau et al., 1992; Mauclère et al., 1993, 1995], raising the possibility that HTLV-II or a related retrovirus also may have been endemic in Africa for a long period of time. This hypothesis was based primarily on the detection of HTLV-II antibodies in two Pygmy tribes living in remote areas of Zaire and Cameroon, populations considered to be the oldest inhabitants of Central Africa [Gessain et al., 1995; Goubau et al., 1993; Froment et al., 1993]. However, very few data are available on the molecular structure of the HTLV-II isolates from Africa. Recently, an HTLV-II subtype a virus was isolated from a female sex worker in Ghana, West Africa [Igarashi et al., 1993], suggesting the possibility of an imported infection. An HTLV-II subtype b virus closely related to the strains found in Paleo-Amerindians was recently described in a Zairean patient, but no sociodemographic data were available [Dube et al., 1994]. Thus the presence of indigenous HTLV-II infection in Africa remained a matter of debate.

Epidemiological studies of HTLV-II infection suggest transmission by sharing contaminated needles and by blood transfusion in the Western world and by breastfeeding in the developing world [Lal et al., 1993; Lee et al., 1989; Rios et al., 1994]. Furthermore, some studies are consistent with the hypothesis that heterosexual transmission may play a significant role in the spread of HTLV-II in both environments [Estebanez et al., 1992; Hjelle et al., 1992a; Schwebke et al., 1994; Wiktor et al., 1992]. HTLV-II has not been linked definitively to any disease [Fouchard et al., 1995; Hall et al., 1994; Weiss, 1994], but rare cases of CD8 lymphoproliferations and neuromyelopathies have been reported in association with this virus [Hall et al., 1994; Hjelle et al., 1990, 1992b].

After the discovery of a nuclear Gabonese family (a woman of 34 years and her parents) infected by the same HTLV-II b molecular variant [data reported in a preliminary short note by Gessain et al., 1994], we decided to carry out an extended study of the relatives of these individuals to determine the duration of HTLV-II infection in the family, to investigate the mode of transmissions between the members of this family (all living in the Franceville area in south Gabon), and to characterize further the molecular HTLV-II variant present in this region. We report the epidemiological and serological and/or molecular data from 41 members of this family with seven individuals infected by HTLV-II and five by HTLV-I. These data are the first evidence of an intrafamilial transmission of HTLV-II in Africa. Furthermore, the presence of a new HTLV-II subtype b molecular variant for at least 60 years in this family suggests that this virus has been in this area of the African continent for a long time.

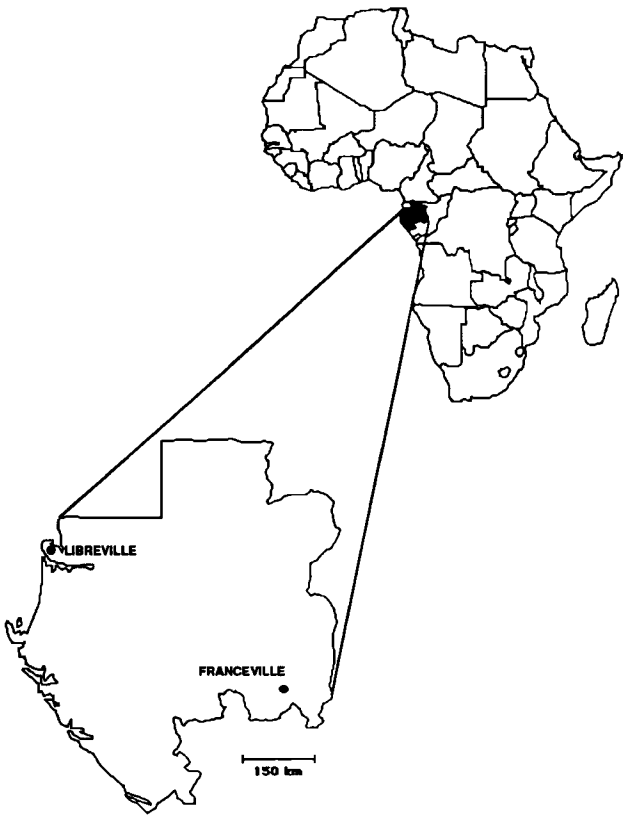

Fig. 1. Map of Gabon, Central Africa, with location of Franceville area where this study took place.

MATERIALS AND METHODS

Area and Study Population

Gabon occupies 270,000 square kilometers and is located in the Gulf of Guinea near the equator. Tropical forest covers three-quarters of the territory. The population was estimated to be 1,273,000 consisting of >40 ethnic groups. Gabon was formerly a French colony and obtained independence in 1960. The district of Franceville is a part of the province of Haut-Ogooué located in southeast Gabon near the border with Congo (Fig. 1). This district has a population of ~55,000, more than half living in the city of Franceville, the semirural capital of the province.

During a serological study on HTLV-I and HIV-1 co-infections carried out in August 1993, a 60-year-old woman (index case PH226PM) was found to have a Western blot (WB) pattern suggestive of an HTLV-II infection (high reactivity against p24, rgp21, and K55 peptide) [Gessain et al., 1994]. A family survey was subsequently conducted during November 1993 to search for HTLV-II infection among her relatives. The family members lived in several villages around

Franceville located southeast of Gabon and in Libreville, the capital of Gabon.

Informed consent was obtained from all subjects studied and parents of minors. They underwent a medical examination, and biological tests were carried out as well as treatment for any medical disorders detected according to the local medical facilities.

Collection of Biological Specimens

Heparinized blood specimens were obtained from the relatives of the index case during medical examination and then taken immediately to the International Center for Medical Research (Franceville) where serum and/or plasma samples were frozen and stored at −20°C until used. Peripheral blood mononuclear cells (PBMCs) were separated by Ficoll-Hypaque sedimentation and stored in liquid nitrogen. All specimens were transported at the end of the survey to the laboratory at the Pasteur Institute in Paris for serological and molecular analysis.

HTLV Serological Assays

All sera were tested by an enzyme-linked immunosorbent assay (ELISA) (Diagnostic Biotechnology, Singapore), a particle agglutination assay (PA, Fujirebio, Japan), and an indirect immunofluorescence assay (IFA), which uses HTLV-I (MT2) and HTLV-II (C19) producing cell lines. WB tests (Diagnostic Biotechnology 2.3, Singapore) containing HTLV-I disrupted virions enriched with a gp21 recombinant protein (reacting with both HTLV-I and HTLV-II) plus two gp46 polypeptides, specific either for HTLV-I (MTA1) or for HTLV-II (K55) were also performed on all sera [Buckner et al., 1992]. A specimen was considered positive for HTLV-II if it reacted in WB with p24, rgp21, and K55 peptide. It was considered as HTLV-I seropositive if it reacted with at least p19, p24, rgp21, and MTA1. Specimens reacting with only gag encoded proteins p19 and/or p24 and/or p26-p28, but without reactivity against rgp21 and to one (or two) of the specific peptides (MTA1-K55), were considered as sero-indeterminate. A sample with no reactivity on the WB was labelled as negative. HTLV-I/II antibody titers were determined for all HTLV-I/II seropositive individuals using IFA on MT2 and C19 cells and particle agglutination.

Polymerase Chain Reaction Assay

The polymerase chain reaction (PCR) was carried out in a DNA thermocycler (Cetus) as previously described [Gessain et al., 1992]. Briefly, high molecular weight DNA was extracted from uncultured PBMCs. Mixtures were made in a special room physically separated from the laboratory. Each initial reaction contained 1.5 μg of DNA, 0.2 mM of dNTPs mix, 10 μl of a 10× reaction buffer, 0.2 mM of each oligonucleotide primer, and 2.5 units of Taq DNA Polymerase (Perkin Elmer Cetus, Branchburg, NJ, USA) in a total volume of 100 μl. Following denaturation at 94°C for 5′, the reaction mixtures containing DNA were cycled 35 times at 94°C 1′, 56° 1′, and 72°C 2′. An extension of 2 seconds per cycle was performed.

A seminested PCR was carried out as described above on the DNA of uncultured PBMCs. For the first PCR run, the outer primer set AGP1 (5080–6000) and WH2 (6641–6659) was used. Two μl volumes of this first PCR run were then transferred to 98 μl of the second PCR mix, which included the inner primer set AGP2 (5110–5132) and WH2, which amplified a fragment of 1508 base pairs (nucleotide 5133–6640 of MO prototype), including 55 bases of the *pol* gene, and the entire *env* gene (1462 base pairs). We also amplified from the same DNA of uncultured PBMCs, by a seminested PCR, a fragment of 519 bp of the *env/pX* region (nucleotide 6481–6969 of MO prototype), using as outer primer set; WH1-WH3 and as inner set; ETH403 and WH3.

Molecular Cloning and Sequencing of PCR Products

After digestion by EcoRI and Not 1 restriction enzymes (Boehringer), the DNA amplified by the two oligonucleotide sets AGP1-WH2, ET403-WH2 (which contained Not1 and EcoR1 restriction sites on their 5′ and 3′ end, respectively) were cloned into the Not1 and EcoR1 restriction sites of a Bluescript vector and then transformed into HB101 competent cells (Gibco, France). Recombinant clones were screened by hybridization under high stringency conditions with the ^{32}P end-labeled oligoprobe HTLV-II PR (5′ GTCATATTGTTTGGCCCCCTGTATCCTCCGC 3′). Nucleotide sequences were determined using the dideoxynucleotide chain-termination method. The EMBL nucleotide sequence database accession number is Z47788 for the HTLV-II JPS *env* gene.

Dendrogram and Phylogenetic Analysis

Several steps were carried out in order to derive phylogenetic trees from the original set of sequences and evaluate their accuracy. Multiple alignment of the sequences was carried out using the Clustal V program [Higgins et al., 1992]. The resulting aligned sequences were submitted to different programs of the PHYLIP package version 3.52c (Joseph Felsenstein, University of Washington). In order to test the reliability of the final tree topology, the "bootstrap" technique was used. For this purpose the SEQBOOT program was carried out to generate 100 data sets that are random resampled versions of the previously aligned sequences. Two different methods were carried out to construct phylogenetic trees: the Maximum Parsimony method with the DNAPARS program and the Neighbor-Joining (NJ) with the NEIGHBOR program [Saitou et al., 1987]. In order to apply the latter methods, distance matrices from each of the 100 replicated data sets were first computed using the DNADIST program with the Kimura 2-parameters model. The obtained matrices were then used as input to the NEIGHBOR program using the Neighbor-Joining. For both Maximum Parsimony and Neighbor-Joining methods, a consensus tree was constructed using the CONSENSE program with

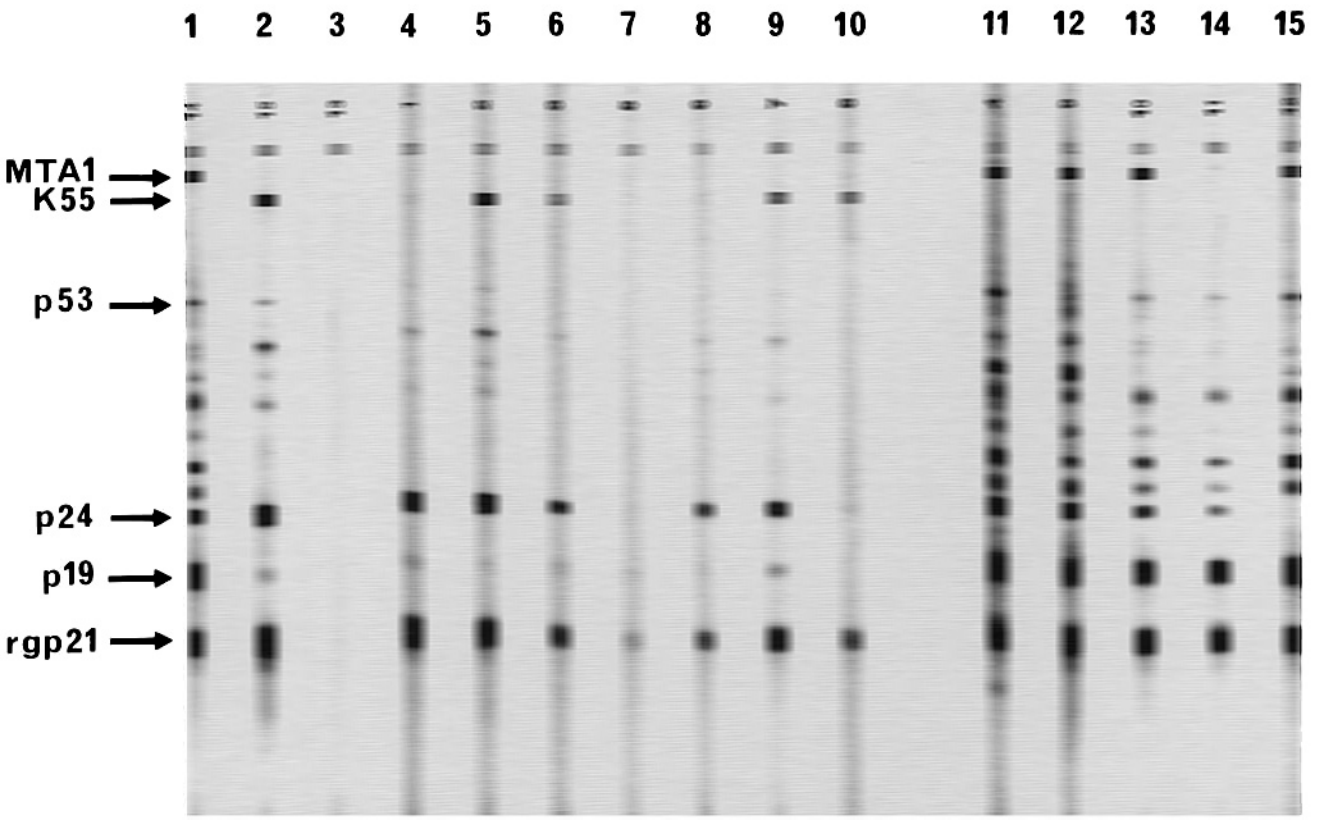

Fig. 2. Recombinant Western blot patterns of selected sera of the JPS family using the WB HTLV-I/II 2.3 from Diagnostic Biotechnology. **Lane 1:** HTLV-I positive control, **lane 2:** HTLV-II positive control, **lane 3:** HTLV-I/II negative control. **Lane 4:** serum from the index case. **Lanes 5–10:** sera from 6 other HTLV-II seropositives. **Lanes 11–15:** sera of 5 individuals showing an HTLV-I WB pattern.

the "majority rule" criteria. In these consensus trees, the branch length has no relationship to the number of nucleotide substitutions.

RESULTS

Description of Families and Serological Analysis

Serological analysis was carried out in 41 subjects belonging to two families (Figs. 2 and 3, Table I). The index case (PH226PM) had been married twice. Among her five children, her only daughter from her first marriage was found HTLV-II positive. Four other children from the second marriage were HTLV negative. The first husband of the index case, i.e., the father of the infected daughter, lives in Congo and was not available for testing. Consequently, the study concentrated on the index case family, in which a 62-year-old sister (PH228MS) and her husband (GAB257NM) were found HTLV-I seropositive (Table I, Fig. 2 and 3). However, the index case revealed that she had had sexual relations with her second husband (PH224JPS) before she was married to the first. Thus the search for HTLV-II seropositives was directed toward the family of the second husband (PH224JPS), who was 58 years of age. This man had had successively four wives: the first one (GAB274) had an indeterminate WB pattern (p19, p26, p28, p32, p36 without reactivity against p24, rgp21, and MTA1 and K55 peptides, not shown in Fig. 2) and was HTLV-I/II negative by PCR; the second was the index case (PH226PM), the third (GAB287RM) was HTLV-II seropositive, but none of her four living children was seropositive. JPS had three living sisters sharing the same father and mother (Fig. 3). One of the sisters (GAB229JM; 56 years of age), was HTLV-II seropositive and among her six children from three different husbands, only one male of 21 years old (GAB290TO) from her first marriage was HTLV-II seropositive. A second sister (GAB227MN) (aged 48) of JPS was also HTLV-II infected (faint seroreactivity on WB but positive by nested PCR).

None of these HTLV-II seropositive individuals had had close contacts with inhabitants of Europe or the Americas. They had never been transfused, they were not sex workers, and they had not been exposed to intravenous drugs. As seen in Figures 2 and 3 and in Table I, three other members of this family were HTLV-I seropositive (GAB299PN, GAB300PM, and GAB294PA). The six individuals with a clear HTLV-II WB pattern had higher antibodies titers by IFA on C19 than on MT2 cells, whereas the opposite was true for

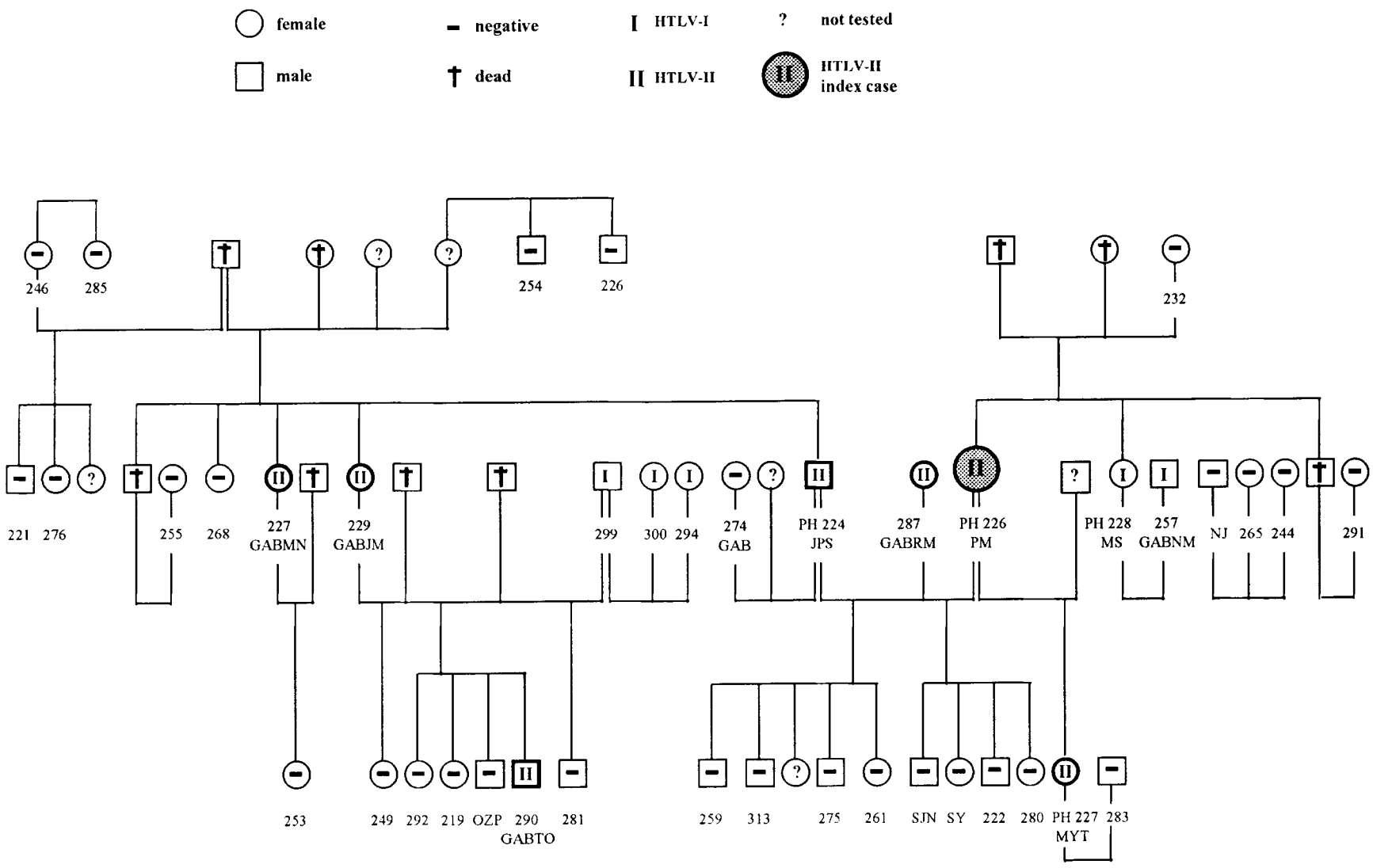

Fig. 3. Three-generation pedigree of the JPS family with HTLV-II and HTLV-I infections from Franceville area, Gabon, Central Africa.

TABLE I. HTLV I/II Antibody Status, Polymerase Chain Reaction (PCR) on Peripheral Blood Mononuclear Cells (PBMCs) for HTLV-I and HTLV-II Seropositive Subjects of the JPS Family From Franceville, Gabon, Central Africa*

Sample	Sex/age	ELISA	PA titer	IF MT2 titer	IF C19 titer	WB	PCR
PH 224 JPS	M/58	+	1024	40	320	II	IIb
PH 226 PM	F/60	+	8192	80	640	II	IIb
PH 227 MYT	F/34	+	256	40	320	II	IIb
PH 228 MS	F/62	+	2048	640	160	I	I
GAB 227 MN	F/48	+	1024	20	10	IIf	IIb
GAB 229 JM	F/56	+	256	20	640	II	nd
GAB 287 RM	F/42	+	1024	40	320	II	nd
GAB 290 TO	M/21	+	290	20	160	II	II
GAB 299 PM	M/50	+	8192	5120	640	I	I
GAB 300 PK	F/45	+	4096	320	40	I	nd
GAB 294 PA	F/49	+	4096	1280	80	I	nd
GAB 257 NM	M/65	+	1024	80	10	I	nd

*Abbreviations: PA: particle agglutination, IF: immunofluorescence, WB: Western blot, PCR: polymerase chain reaction, f: faint seroreactivity, nd: not done.

the five individuals with an HTLV-I WB pattern (Table I). Only, GAB227MN who exhibited a very faint HTLV-II WB seroreactivity had similar IFA titers on C19 and on MT2. All this family belonged to the Bahoumbou ethnic group.

Nucleotide Sequence Analysis

PCR was carried out on DNA extracted from uncultured PBMCs of 24 individuals including five HTLV-II seropositive (including one with a faint WB seroreactivity: GAB 227MN), two HTLV-I seropositive, and 17 HTLV seronegative or seroindeterminate individuals having a parent, a sister, or a brother HTLV-II seropositive. The five HTLV-II seropositive subjects were found to be positive by nested PCR using the HTLV-II specific primers pair ET403-WH2 as inner set that amplify a fragment of 172 nucleotides from the gp21 *env* gene. Furthermore, this fragment was cloned and sequenced from four out of these five seropositive individuals and revealed a new HTLV-II molecular variant identical among all the individuals, which was closely related to but different from the HTLV-II subtype b prototypes (NRA and G12), seven (4.1%) and five (2.9%) bases substitutions, respectively. It was more distantly related to the subtype a (MO) prototype (13 bases substitution, 7.6%) (see Table II). A fragment consisting of 1,508 base pairs (encompassing all of the *env* gene) was amplified, cloned, and sequenced from the DNA of uncultured PBMCs from the second husband (JPS) of the index case by seminested PCR with the AGP2-WH2 inner primer set. Comparison of this sequence with HTLV-II subtype a (MO) [Shimotohno et al., 1985] and subtype b (NRA and G12) [Lee et al., 1993; Pardi et al., 1993] prototype strains demonstrated a close homology with the subtype b group. Thus in the total *env* gene (1,462 base pairs), HTLV-II JPS exhibited only 2.3% (33 substitutions) nucleotide divergence from G12 and 2.3% (33 substitutions) divergence from NRA, but 5.2% (76 bases) divergence from the HTLV-II MO subtype a prototype (Fig. 4). This divergence in sequence, as compared to HTLV-II subtype b prototypes, was considered unusual as this gene region is remarkably conserved [Hall et al., 1993b]. Comparison at the amino acid level (aa) of the entire JPS env protein revealed only a 2% (10 aa) divergence with NRA and 1.4% (7 aa) with G12, whereas the variability was as expected higher (17 aa, 3.5%) with MO strain. A smaller fragment of the *env* gene (WH1-WH2) was also sequenced from the index case DNA (data not shown) and revealed only one base different from the JPS strain confirming on a larger sequence (589 bp) the presence of a similar new variant of HTLV-II subtype b in the couple. The portion of the pX region located between the end of the *env* gene and the splice junction for the *Tax/rex* (6644–7215:571 bases pairs) was quite conserved exhibiting only 1.4% of nucleotide divergence to NRA strain but 6.6% with MO strain.

A dendrogram comparing the nucleotide sequences of this *env* fragment (6052–6640) showed that this new Gabonese HTLV-II clustered clearly in the subtype b group (Fig. 5A) being, however, the most divergent HTLV-II subtype b yet described. Phylogenetic trees were constructed with two different methods (NJ and DNAPars) on this same *env* region coding for the gp21 (Fig. 5B). In both cases, the topology of the three trees was very similar, showing the existence of two molecular clusters of HTLV-II genotypes with the JPS isolate always included within the subtype b cluster. The similar topology and the bootstrap values of the trees allowed a reliable interpretation of the two clusters. In one individual infected by HTLV-I (GAB294PA), cloning and sequencing of 522 bp encompassing most of the gp21 and the carboxy-terminus of the gp46, as previously described [Gessain et al., 1992], revealed an HTLV-I molecular variant closely related to the Central African HTLV-I isolates from Zaïre exhibiting 2.5% of nucleotide substitutions as compared to the cosmopolitain group of HTLV-I strains.

DISCUSSION

A virological investigation undertaken in a Gabonese family living in a tropical forest region of south-

TABLE II. Nucleotide Sequences of a 172-bp Region of gp21^{env} of HTLV-II Strains From Central Africa (Gabon) as Compared to Published HTLV-II Strains*

Geographical origin	Name	6469	6472	6478	6514	6518	6520	6532	6533	6553	6568	6571	6577	6580	6601	6603	6611	6619	6624	6628	6633	HTLV-II Subtype
US	MO	A	G	A	T	G	T	C	G	T	C	G	T	A	T	A	T	C	C	A	T	
US IVDA	IIAWH2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	HTLV IIA
US IVDA	IIAWH2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
US Amerindian	408N	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
US Amerindian	1358N	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
US Amerindian	MSA1bp	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Italy IVDA	Md	—	—	—	C	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Italy IVDA	Bo	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Gabon	GJPS	T	—	G	—	—	C	**T**	**C**	C	**T**	A	—	G	**C**	—	G	**T**	—	G	—	HTLV IIB
	GPM	T	—	G	—	—	C	**T**	**C**	C	**T**	A	—	G	**C**	—	G	**T**	—	G	—	Gabonese
	GMYT	T	—	G	—	—	C	**T**	**C**	C	**T**	A	—	G	**C**	—	G	**T**	—	G	—	Variant
	GMN	T	—	G	—	—	C	**T**	**C**	C	**T**	A	—	G	**C**	—	G	**T**	—	G	—	
US	RNA	T	—	G	—	A	C	T	—	C	—	A	—	G	—	G	G	—	A	G	—	
Panama Amer.	G12	T	A	G	—	—	C	T	—	C	—	A	—	G	—	—	G	—	—	—	—	
US IVDA	IIBWH6	T	—	G	—	—	C	T	—	C	—	A	C	G	—	G	G	—	—	G	—	
US IVDA	IIBWH7	T	—	G	—	—	C	T	—	C	—	A	—	G	—	G	G	—	—	G	—	
US Amerindian	60405N	T	—	G	—	—	C	T	—	C	—	A	—	G	—	G	G	—	—	G	—	
US Amerindian	72969N	T	—	G	—	—	C	T	—	C	—	A	—	G	—	G	G	—	—	G	—	HTLV IIB
US Amerindian	130P	T	—	G	—	—	C	T	—	C	—	A	—	G	—	G	G	—	—	G	—	Paleo-Indian
US Amerindian	3526P	T	—	G	—	—	C	T	—	C	—	A	—	G	—	G	G	—	—	G	—	Type
US Amerindian	1457P	T	—	G	—	—	C	T	—	C		A	—	G	—	G	G	—	—	G	—	
US Amerindian	52580P	T	—	G	—	—	C	T	—	C	—	A	—	G	—	G	G	—	—	G	—	
US Amerindian	3564P	T	—	G	—	—	C	T	—	C	—	A	—	G	—	G	G	—	—	G	—	
US Amerindian	1214P	T	—	G	—	—	C	T	—	C	—	A	—	G	—	G	G	—	—	G	—	
US Amerindian	62245P	T	—	—	—	—	C	T	—	C	—	A	—	G	—	G	G	—	—	G	—	
Italy IVDA	Gu	T	—	G	—	—	C	C	—	C	—	A	—	G	—	G	G	—	—	G	—	
Italy IVDA	Va	T	—	G	—	—	C	C	—	—	—	A	—	G	—	G	G	—	—	—	—	
Cameroun Pygmy	Pygcaml	T	A	G	—	—	C	C	—	C	—	A	—	G	—	G	G	—	—	—	C	

*This includes subtype a (MO) prototype, subtype b (NRA) prototype, and the 22 other HTLV-II isolates including 7 subtype a and 15 subtype b. Nucleotide substitutions with respect to the corresponding region in the prototypic MO sequence are shown. The four members of the Gabonese family studied harbor the same HTLV-II subtype b nucleotide variant. IVDA: Intravenous drug abusers. Nucleotides printed in boldface correspond to substitutions found only in the four HTLV-II strains from Gabon.

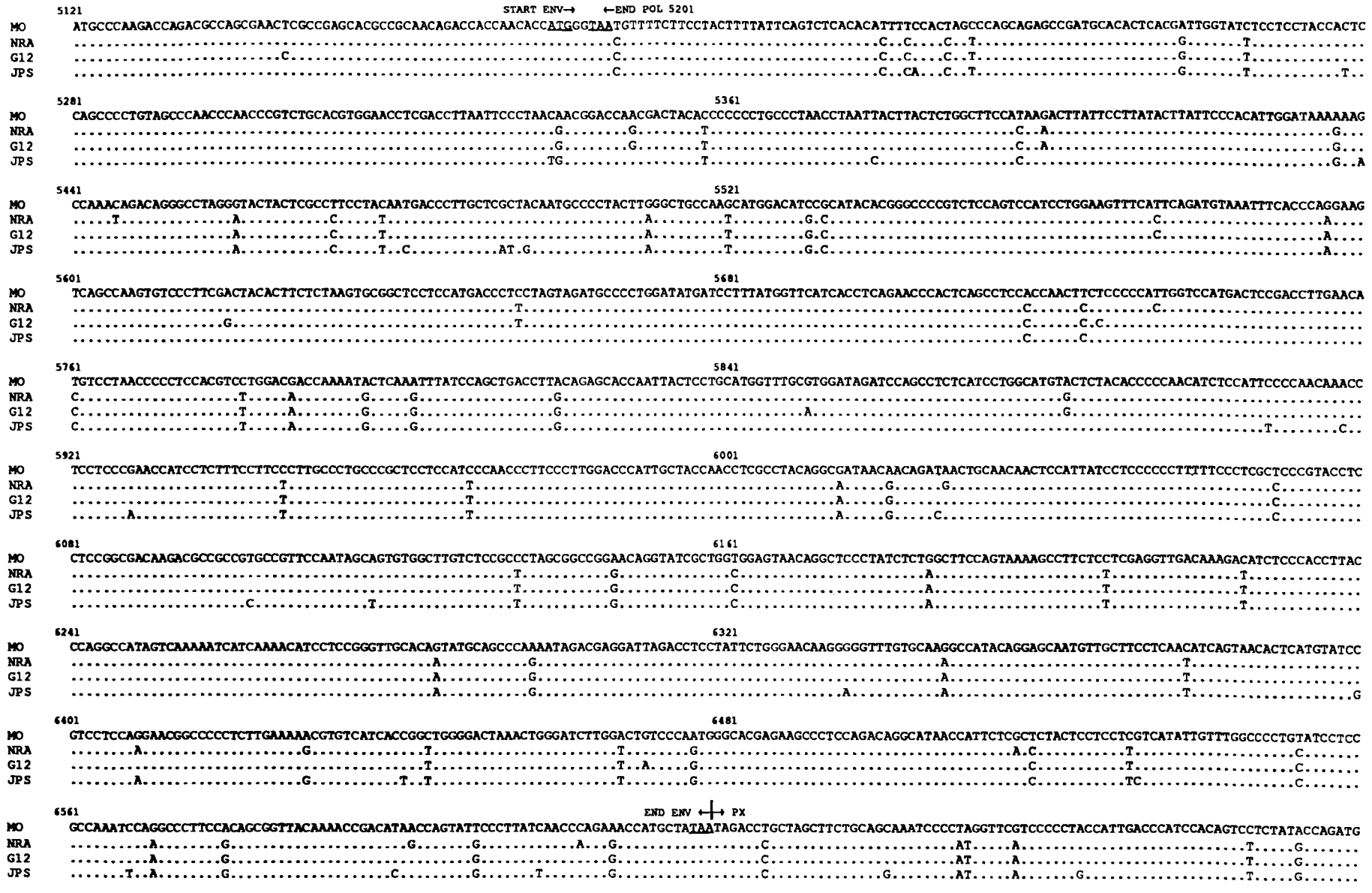

Fig. 4. Nucleotide sequence of 1,600 base pairs encompassing all the *env* gene(1462) of the JPS strain. The sequences selected as prototypes are MO (HTLV-IIa) [Shimotohno et al., 1985] and G12 [Lee et al., 1993], NRA (HTLV-IIb) [Pardi et al., 1993].

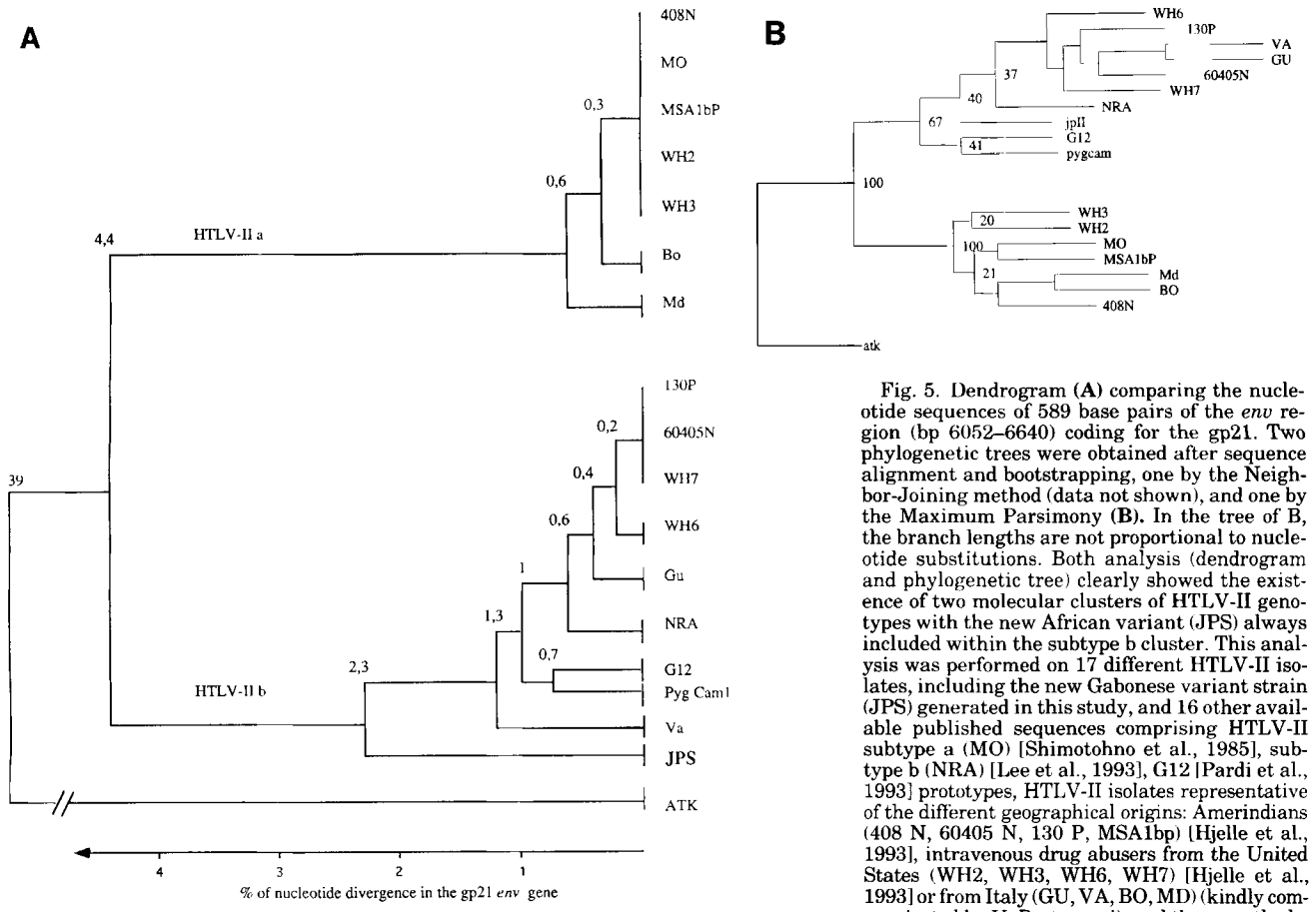

Fig. 5. Dendrogram (**A**) comparing the nucleotide sequences of 589 base pairs of the *env* region (bp 6052–6640) coding for the gp21. Two phylogenetic trees were obtained after sequence alignment and bootstrapping, one by the Neighbor-Joining method (data not shown), and one by the Maximum Parsimony (**B**). In the tree of B, the branch lengths are not proportional to nucleotide substitutions. Both analysis (dendrogram and phylogenetic tree) clearly showed the existence of two molecular clusters of HTLV-II genotypes with the new African variant (JPS) always included within the subtype b cluster. This analysis was performed on 17 different HTLV-II isolates, including the new Gabonese variant strain (JPS) generated in this study, and 16 other available published sequences comprising HTLV-II subtype a (MO) [Shimotohno et al., 1985], subtype b (NRA) [Lee et al., 1993], G12 [Pardi et al., 1993] prototypes, HTLV-II isolates representative of the different geographical origins: Amerindians (408 N, 60405 N, 130 P, MSA1bp) [Hjelle et al., 1993], intravenous drug abusers from the United States (WH2, WH3, WH6, WH7) [Hjelle et al., 1993] or from Italy (GU, VA, BO, MD) (kindly communicated by U. Bertazonni), and the recently described HTLV-II b (Pyg Cam1) from a pygmy living in a remote area of Cameroon [Gessain et al., 1995]. The HTLV-I ATK isolate was used as outgroup to root the phylogenetic trees.

eastern Gabon revealed the existence of HTLV-II infection in seven members (out of the 41 studied) with the presence of the same HTLV-II molecular variant in four out of the four individuals investigated. Molecular analysis (based on the study of the entire *env* gene and parts of the pol and pX regions) confirmed that this African HTLV-II was the most divergent HTLV-II subtype b known. The infection of a man, aged 58, and of two of his sisters, aged 56 and 48, by the same variant further suggested the presence of this African HTLV-II in this family for at least two generations.

At about 1930, the region of Franceville was a remote forest area hardly visited by foreigners. Moreover, intravenous drug abuse was nonexistant at that time and even now is extremely rare in the area. Blood transfusion has been practiced only very recently. Consequently, transmission from mother to child and by sexual intercourse can be considered as the major routes of transmission of this new HTLV-II isolate. In the family studied, two HTLV-IIb infected mothers had infected children. This represents the first evidence of mother to child transmission of HTLV-II in Africa, most probably by breast milk from HTLV-II infected mothers, milk known to contain HTLV-II genomic sequences [Gallo et al., 1993; Heneine et al., 1992; Kaplan et al., 1992]. In the area of Franceville, infants are breastfed for at least 18–24 months, suggesting that mother-to-child transmission of HTLV-II prevailed during this period. Only one previous case of mother-to-child transmission of HTLV-II based on molecular evidence has previously been reported in a Mexican sex worker mother whose child had been breastfed for 4 years [Lal et al., 1993].

Several studies have suggested that heterosexual transmission might play a significant role in the epidemiology of HTLV-II [Estebanez et al., 1992; Hjelle et al., 1992a; Schwebke et al., 1994; Wiktor et al., 1992]. Individuals seropositive for syphilis or HSV-2, sexual partners of IVDA, or non-IVDA sex workers were more frequently HTLV-II-seropositive. In the present study, serologic and PCR analyses suggested that a man (JPS) might have infected two of his three spouses, with the same HTLV-IIb molecular variant having a 100% identical sequence in the gp21 encoding env region. This man (JPS) had a brief relationship (3 years) and no children with his first wife who was not infected by HTLV-II, but he had five children with his infected third wife with a relationship lasting 15 years. Thus sexual transmission of HTLV-II, as is the case for

HTLV-I, appears to be efficient from male to female [Mueller et al., 1990]. This is also supported by the high HTLV-II infection rates reported for old females in southern American Indian groups [Maloney et al., 1992].

From a molecular point of view, it was surprising to find a putative genuine African HTLV-II to be closely related to the HTLV-II subtype b, believed, up to now, to be present mainly in the Paleo-Indians of the Americas. Recent molecular data indicate that the HTLV-II genetic variability between strains of the same subtype is extremely low [Dube et al., 1993; Gessain et al., 1995; Hall et al., 1993b; Ijichi et al., 1993]. A nucleotide divergence of only 0 to 0.4% (>1,000 bp of the *env* gene) was observed between HTLV-IIb of different Indian groups in North, Central, or South America with most probably no contacts between them for several thousand years [Ijichi et al., 1993; Hall et al., 1993b; Pardi et al., 1993]. Furthermore, preliminary data from Hall et al. [1993a] described the presence in Mongolia of a typical HTLV-II subtype a close to the subtype a strains present in the Americas. Such findings support the hypothesis that the in vivo genetic drift might be much lower for HTLV-II than for HTLV-I for which an estimate of a 0.5–1% nucleotide divergence (in the *env* and *pol* gene) over 1,000 years of evolution has been proposed [Gessain et al., 1994a,b; Hall et al., 1993a; Yanagihara, 1994]. It is interesting to note that the four African HTLV-II subtype b strains described to date were found in relatively remote areas of Gabon, Zaïre, and Cameroon [E. Delaporte, pers. comm., 1994; Dube et al., 1994; Gessain et al., 1995], suggesting that genuine HTLV-II-b was present in Africa over a long period of time. In contrast, the only two African HTLV-IIa infections were reported from sex workers living in large towns in Ghana and Cameroon, suggesting a possibly imported infection by an HTLV-II of subtype a in West/Central Africa [Igarashi et al., 1993; Mauclère et al., 1995].

The finding of five individuals infected by HTLV-I in this family confirmed that this area is highly endemic for HTLV-I [Delaporte et al., 1992]. Further search for other genuine African HTLV-II infections should be extended in various Pygmy populations [Gessain et al., 1995] and other ancient isolated African ethnic groups. Family investigations, will be crucial for understanding the origin, evolution, and modes of dissemination of these primate T lymphotropic viruses in Africa.

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REFERENCES

Biglione M, Gessain A, Quiruelas S, Fay O, Taborda MA, Fernandez E, Lupo S, Panzita A, de Thé G (1993): Endemic HTLV-II infection among Tobas and Matacos Amerindians from North Argentina. Journal of Acquired Immune Deficiency Syndromes 6:631–633.

Bonis J, Verdier M, Dumas M, Denis F (1994): Low Human T cell Leukemia virus type II seroprevalence in Africa. Journal of Infectious Diseases 169:225–227.

Buckner C, Roberts CR, Foung SKH, Lipka J, Reyes GR, Hadlock K, Chan L, Congora-Biachi RA, Hjelle B, Lal R (1992): Immune responsiveness to the immunodominant recombinant envelope epitopes of human T lymphotropic virus types I and II in diverse geographic populations. Journal of Infectious Diseases 166:1160–1163.

Chen YA, Jang YJ, Kanki PJ, Yu QC, Wang JJ, Montali RJ, Samuel KP, Papas TS (1994): Isolation and characterization of Simian T-cell leukemia virus type II from new world monkeys. Journal of Virology 68:1149–1157.

Delaporte E, Monplaisir N, Louwagie J, Peeters M, Martin-Prével Y, Louis JP, Trebucq A, Bedjabaga I, Ossari S, Honore C, Larouzé B, d'Auriol L, Van der Groen, Piot P (1992): Prevalence of HTLV-I and HTLV-II infection in Gabon, Africa: Comparison of the serological and PCR results. International Journal of Cancer 49:373–376.

Dube KD, Sherman MP, Saksena N, Bryz-Gornia V, Mendelson J, Love J, Arnorld CB, Spicer T, Dube S, Glaser J, Williams AE, Nishimura M, Jacobsen S, Ferrer JF, del Pino N, Quiruelas S, Poiesz BJ (1993): Genetic heterogeneity in human T-cell leukemia/lymphoma virus type II. Journal of Virology 67:1175–1184.

Dube KD, Dube S, Erensoy S, Jones B, Bryz-Gornia V, Spicer T, Love J, Saksena N, Lechat MF, Shrager DI, Dosik H, Glaser J, Levis W, Blattner W, Montagna R, Blumberg B, Poiesz BJ (1994): Serological and nucleic acid analysis for HIV and HTLV infection on archival human plasma samples from Zaire. Virology 202:379–389.

Estabanez P, Varela JM, Rua-Figueroa M, Sarasqueta C, Contreras G, Casado C, Palacios V, Garcia A, Fitch K, Najera R (1992): The presence of HTLV-I/II in non-intravenous drug using spanish prostitutes. International Journal of STD and AIDS 3:62–63.

Ferrer JF, Del Pino N, Esteban E, Sherman MP, Dube S, Dube DK, Basombrio MA, Pimentel E, Segovia A, Quirulas S, Poiesz BJ (1993): High rate of infection with the human T-cell leukemia retrovirus type II in four Indian populations of Argentina. Virology 197:576–584.

Fouchard N, Flageul B, Bagot M, Avril M-F, Hermine O, Sigaux F, Merle-Beral H, Troussard X, Delfraissy J-F, de Thé G, Gessain A (1995): Lack of evidence of HTLV- I/II infection in T CD8 malignant or reactive lymphoproliferative disorders in France: A serological and/or molecular study of 169 cases. Leukemia (in press).

Froment A, Delaporte E, Dazza M-C, Larouzé B (1993): HTLV-II among Pygmies from Cameroon. AIDS Research and Human Retroviruses 9:707.

Gallo D, Petru A, Yeh ET, Hanson CV (1993): No evidence of perinatal transmission of HTLV-II. Journal of Acquired Immune Deficiency Syndromes 6:1168–1170.

Gessain A, de Thé G (1994a): Molecular Epidemiology of HTLV-I and II. In Takatsuki N (ed): "Adult T-cell Leukemia." Oxford: University Press, pp 139–161.

Gessain A, Gallo RC, Franchini G (1992): Low degree of human T-cell leukemia virus type I genetic drift in vivo as a means of monitoring viral transmission and movement of ancient human populations. Journal of Virology 66:2288–2295.

Gessain A, Fretz C, Koulibaly M, Boudret ML, Bah A, Raphael M, de Thé G, Fournel JJ (1993): Evidence of HTLV-II infection in Guinea, West Africa. J Acquired Immune Deficiency Syndromes 6:324–325.

Gessain A, Tuppin P, Kazanji M, Cosnefroy JY, Georges-Courbot MC, Georges AJ, de Thé G (1994): A distinct molecular variant of HTLV-IIb in Gabon, Central Africa. AIDS Research and Human Retroviruses 6:753–755.

Gessain A, Mauclère P, Froment A, Biglione M, Le Hesran JY, Tekaia F, Millan J, de Thé G (1995): Isolation and molecular characterization of a human T lymphotropic virus type II-subtype b, from a healthy pygmy living in a remote area of Cameroon: An ancient origin for HTLV-II in Africa. Proceedings of the National Academy of Sciences USA 92:4041–4055.

Goubau P, Desmyter J, Ghesquire J, Kasereka B (1992): HTLV-II among Pygmies. Nature 359:201.

Goubau P, Liu H-F, de Lange GG, Vandamme A-M, Desmyter J (1993): HTLV-II seroprevalence in Pygmies across Africa since 1970. AIDS Research and Human Retroviruses 9:709–713.

Goubau P, Van Brussel M, Vandamme A-M, Liu H-F, Desmyter J (1994): A primate T-lymphotropic virus, PTLV-I, different from human T-lymphotropic viruses types I and II, in a wild-caught baboon *(Papio hamadryas)*. Proceedings of the National Academy of Sciences USA 91:2848–2852.

Hall WW, Takahashi H, Liu C, Kaplan MH, Scheewing O, Ijichi S, Nagashima K, Gallo RC (1992): Multiple isolates and characteristics of Human T-cell leukemia virus type II. Journal of Virology 66:2456–2463.

Hall WW, Zhu SW, Horal P, Furuta Y, Zagaany G, Vahlne A (1993a): HTLV-II infection in Mongolia (abstract 2). In Program and abstracts from the 6th international conference on human retrovirology, Absecon, New-Jersey. AIDS Research and Human Retroviruses 10:443.

Hall WW, Takahashi H, Zhu SW, Ijichi S (1993b): Human T cell leukemia virus type II (HTLV-II). In Levy JA, Weiss RA, Neu HC (eds): "Frontiers in infectious Diseases: focus on HIV." London: Churchill Livingston Press, pp 156–172.

Hall WW, Kubo T, Ijichi S, Takahashi H, Zhu SW (1994): Human T cell leukemia/lymphoma virus type II (HTLV-II): Emergence of an important newly recognized pathogen. Seminars in Virology 5:165–178.

Heneine W, Woods T, Green D, Fukuda K, Giusti R, Castillo L, Armien B, Gracia F, Kaplan JE (1992): Detection of HTLV-II in breast milk of HTLV-II infected mothers. Lancet 340:1157–1158.

Higgins DG, Bleasby AJ, Fuchs R (1992): CLUSTAL V: improved software for multiple sequence alignment. CABIOS 8:189–191.

Hjelle B, Mills R, Swenson S, Mertz G, Key C, Allen C (1990): Incidence of hairy cell leukemia, mycosis fungoides and chronic lymphocytic leukemia in first known HTLV-II endemic population. Journal of Infectious Diseases 163:435–440.

Hjelle B, Cyrus S, Swenson SG (1992a): Evidence for sexual transmission of human T lymphotropic virus type II. Annals of Internal Medicine 116:90–91.

Hjelle B, Appenzeller O, Mills R, Alexander S, Torrez-Martinez N, Jahnker R, Ross G (1992b): Chronic neurodegenerative disease associated with HTLV-II infection. Lancet 339:645–646.

Hjelle B, Zhu SW, Takahashi H, Ijichi S, Hall WW (1993): Endemic human T cell leukemia virus type II infection in Southwestern US Indians involves two prototype variants of virus. Journal of Infectious Diseases 168:737–740.

Igarashi T, Yamashita M, Miura T, Osei-Kwasi M, Aysi NK, Shiraki H, Kurimura T, Hayami M (1993): Isolation and genomic analysis of human T lymphotropic virus type II from Ghana. AIDS Research and Human Retroviruses 9:1039–1042.

Ijichi S, Zaninovic V, Leon S, Katahira Y, Sonoda S, Miura T, Hayami M, Hall WW (1993): Identification of human T cell leukemia virus type IIb infection in the Wayu, an aboriginal population of Columbia. Japanese Journal of Cancer Research 84:1215–1218.

Kalyanaraman VS, Sarngadharan MG, Robert-Guroff M, Miyoshi I, Blayney D, Golde D, Gallo RC (1982): A new subtype of human T-cell leukemia virus (HTLV-II) associated with a T-cell variant of hairy cell leukemia. Science 218:571–573.

Kaplan JE, Abrams E, Shaffer N, Cannon RO, Kaul A, Krasinski K, Bamji M, Hartley TM, Roberts B, Kilbourne B (1992): Low risk of mother-to-child transmission of human T-lymphotropic virus type II (HTLV-II) in non-breast infants. Journal of Infectious Diseases 166:892–895.

Lairmore Md, Jacobson S, Gracia F, De BK, Castillo L, Larreategui M, Roberts BD, Levine PH, Blattner WA, Kaplan JE (1990): Isolation of human T-lymphotropic virus type 2 from Guayami Indians in Panama. Proceedings of the National Academy of Sciences USA 87:8840–8844.

Lal RB, Gongora-Biachi RA, Pardi D, Switzer WM, Goldman I, Lal AA (1993): Evidence for mother-to-child transmission of Human T Lymphotropic virus type II. Journal of Infectious Diseases 168: 586–591.

Lee HH, Swanson P, Shorty VS, Zack JA, Rosenblatt JD, Chen ISY (1989): High rate of HTLV-II infection in seropositive IV drug abusers from New Orleans. Science 244:471–475.

Lee H, Idler KB, Swanson P, Aparicio JJ, Chin KK, Lax JP, Nguyen M, Mann T, Leckie G, Zanetti A, Marinucci G, Chen ISY, Rosenblatt JD (1993): Complete nucleotide sequence of HTLV-II isolate NRA: Comparison of envelope sequence variation of HTLV-II isolates from U.S. blood donors and U.S. and Italian IV drug users. Virology 196:57–69.

Levine PH, Jacobson S, Elliot R, Cavallero A, Colclough G, Dorry C, Stephson C, Knigge RM, Drummond J, Nishimura M, Taylor ME, Wiktor S, Shaw GM (1993): HTLV-II infection in Florida Indians. AIDS Research and Human Retroviruses 9:123–127.

Liu HF, Vandamme AM, Van Brussel M, Desmyter J, Goubau P (1994): New retroviruses in Human and Simian T-Lymphotropic viruses. Lancet 344:265–266.

Maloney EM, Biggar RJ, Neel JV, Taylor ME, Hahn BH, Shaw GM, Blattner WA (1992): Endemic human T cell lymphotropic virus type II infection among isolated Brazilian Amerindians. Journal of Infectious Diseases 166:100–107.

Mauclère P, Gessain A, Garcia-Calleja JM, Le Hesran JY, Salla R, Rehle T, de Thé G (1993): HTLV-II in African Prostitutes from Cameroon. AIDS 7:1394–1395.

Mauclère P, Mahieux R, Garcia-Calleja JM, Salla R, Tekaia F, Millan J, de Thé G, Gessain A (1995): A new HTLV-II subtype A in an HIV-1 coinfected prostitute from Cameroun, Central Africa. AIDS Research and Human Retroviruses 11:989–993.

Miyoshi I, Yoshimoto S, Fujishita M, Tagushi H, Kubonishi I, Niiya K, Minezawa M (1982): Natural adult T-cell leukemia virus infection in Japanese monkeys. Lancet IIb:658.

Mueller N, Tachibana N, Stuver SO, Okayama A, Ishizaki J, Shishime E, Murai K, Shioiri S, Tsuda K (1990): Epidemiologic perspectives of HTLV-I. In Blattner WA (ed): "Human retrovirology: HTLV." New York: Raven Press, pp 281–293.

Pardi DJ, Switzer WM, Hadlock KG, Kaplan JE, Lal RB, Folks TM (1993): Complete nucleotide sequence of an Amerindian human T-cell lymphotropic virus type II (HTLV-II) Isolate: Identification of a variant HTLV-II subtype b from a Guayami Indian. Virology 67:4659–4664.

Poiesz BJ, Ruscetti FWI, Gazdar AF, Bunn PA, Minua JD, Gallo RC (1980): Detection and isolation of type-C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proceedings of the National Academy of Sciences USA 77:7415–7419.

Rios M, Khabbaz RF, Kaplan JE, Hall WW, Kessler D, Bianco C (1994): Transmission of Human T cell Lymphotropic Virus (HTLV) type II by transfusion of HTLV-I-screened blood products. Journal of Infectious Diseases 170:206–210.

Saitou N, Nei M (1987): The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406–425.

Schwebke J, Calsyn D, Shriver K, Saxon A, Kleyn J, Oluoch-Mitchell E, Olmsted L, Fisher LD, Krone M, Ashley R, Stamm W, Swenson P, Holmes KK (1994): Prevalence and epidemiologic correlate of human T cell lymphotropic virus (HTLV) infection among intravenous drug abusers. Journal of Infectious Diseases 169:962–967.

Shimotohno K, Takahashi Y, Shimizu N, Gojobori T, Golde DW, Chen ISY, Miwa M, Sugimura T (1985): Complete nucleotide sequence of an infectious clone of human T-cell leukemia virus type II: An open reading frame for the protease gene. Proceedings of the National Academy of Sciences USA 82:3101–3105.

Soriano V, Calderon E, Esparza B, Cilla G, Aguilera A, Gutierrez M, Tor J, Pujol E, Merino F, Perez-Trallero E, Leal M, Gonzalez-Lahoz J (1993): HTLV-I/II infections in Spain. International Journal of Epidemiology 22:129–132.

Switzer WM, Pieniazek D, Swanson P, Samdal HH, Soriano V, Khabbaz RF, Kaplan JE, Lal RB, and Heneine W (1995): Phylogenetic relationship and geographic distribution of multiple human T-cell lymphotropic virus type II subtypes. J Virology 69:621–632.

Weiss SH (1994): The evolving epidemiology of Human T Lymphotropic Virus type II. Journal of Infectious Diseases 169:1080–1083.

Wiktor SZ, Cannon RO, Atkinson WL, Lutz B, Hook III EW, Blattner WA, Quinn TC (1992): Infection with Human T lymphotropic virus types I and II in sexually transmitted disease clinics in Baltimore and New Orleans. Journal of Infectious Diseases 165:920–924.

Yanagihara R (1994): Geographic-specific genotypes or topotypes of human T-cell lymphotropic virus type I as markers for early and recent migrations of human populations. Advances in Virus Research 43:147–186.

Zella D, Mori L, Sala M, Ferrante P, Casoli C, Magnani G, Achilli G, Cattaneo E, Lori F, Bertazzoni U (1990): HTLV-II infection in Italian drug abusers. Lancet 336:575–576.